Appendix 5L

PNS Supervisor's Review and Release For 1st and 2nd Trimester Specimens

Tracking Number: CP 001

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I. TITLE

TMS Supervisor's Review and release

II. PRINCIPLE

At the completion of each day's testing, the supervisor at the screening laboratory must review and release the day's data. NAPS supervisor and any qualified CLS who is trained and authorized by the NAPS supervisor are allow to review and release results. Data files from the AutoDELFIA are downloaded to the PC. The data files are put through MultiCalc, the QC software, to calculate results and score controls for determination of run/tray/result status. Software will provide QC plots, trend plots, standard curves, specimen's testing history, and repeat list.

Security is maintained with different access levels to the data base. Staff with Security Level 1for read only can view the run data using the procedures below but cannot make any changes to the data .

III. SPECIMEN COLLECTION AND TYPE

Refer to the Automated Dissociated Enhanced Lanthanide Fluoro Immunoassay System (AutoDELFIA) for Prenatal Screening Using Maternal Serum: 1st Trimester Markers are Pregnancy Associated Plasma Protein-A (PAPPA) and Human Chorionic Gonadotropin (HG1), 2nd Trimester Markers are Human Chorionic Gonadotropin (hCG), Alphafetoprotein (AFP) and Unconjugated Estriol (uE3) protocol.

IV. EQUIPMENT AND SUPPLIES

Refer to the protocol named in Section III.

V. REAGENTS

Refer to the protocol named in Section III.

VI. CALIBRATION AND QUALITY CONTROL

Refer to the protocol named in Section III.

VII. PROCEDURES

A. REVIEW AND RELEASE

Power to the supervisor's PC should be on at all times. Review and release of the Prenatal Screening (PNS) runs can be performed at either the NBS or TMS

supervisor's PC.

1. Follow the prompt on the screen to use Ctrl, Alt, Delete to logon.
2. Enter user name and password. Supervisors performing review and release must logon at Level 2. If there is an email message sent to you, the screen goes to the **eMail** screen. New email messages will be generated whenever GDL makes a change to the "assay protocol" in QA to change lot numbers, std concentrations, control limits, etc. Read it, delete it, and click **Exit**.
3. Click **PNS/Result Viewer**. Data Plotter is available, but for screening, your laboratory is using Multicalc.
4. View the screen. The screen shows the map of California and your laboratory icon.
5. Select **Screening Assays** to access screening runs or **Training Assays** to access the runs in the training mode. Select **Assays waiting for review** to access current runs for review and release or **Selected date (read-only)** to view archive runs. Select **Reports** to view/print the local and GDL list of repeats.
6. Click onto the laboratory icon to begin.
   View the screen. The screen shows runs under the **AutoDELFIA Instruments: All AutoDELFIA Assays** window or runs under the **API 300 Instruments: All API 300 Assays** window. (With PNS the API 300 instrument screen is blank.) Click onto AutoDELFIA Instruments for the PNS runs.

   In the AutoDELFIA Instruments screen are the two or three AutoDELFIAs. Only runs for the two or three PNS AutoDELFIAs will be on the screen. Runs for review and release are identified by analyte, the score for the run by MultiCalc, the score for each tray, test date, instrument ID, and run ID.

   Each instrument is a button. If you only want to see the runs from one instrument, click the instrument icon.

7. Click onto the assay bar for review and release of that run.
   a. View the entire run. Information at the top of the screen includes your laboratory icon, instrument ID, test date, run ID, analyte, each tray in the run and the elevator shelf used. There are 12 elevator shelves. Each shelf shows a tray with the 96 well positions.
   b. Click one of four selections, Status, Response, Sample, and Other from the **Well Color Type** window. The selection will determine how to view the 96 well positions. Each selection has it own pull down menu. The chart below shows the choices for each selection.

Well Color Type Choices

	Status	***Response***	***Sample***	***Other***
Result Overall	✓			
NAPS Multicalc	✓			
NAPS Review	✓			
GDL Multicalc	✓			
GDL Review	✓			
Concentration		✓		
Conc. Ratio		✓		
Counts/Volts		✓		
Sample Type			✓	
Probe			✓	
Checkmarks				✓
Positives				✓
Repeats				✓

The default selection is **Status**. The 96 well positions change colors according to the Color Description with each selection.

For Status, use Result Overall for review and release. NAPS Multicalc and Review are more useful when viewing archive data. GDL Multicalc and Review are gray choices (not available).

For Response, only concentration and counts/volts are relevant for PNS.

For Sample, use Probe for PNS. This identifies which of the 4 sample probes was used to transfer the sample from the dilution strip for HG1, PAPPA, and hCG or from collection tube for AFP and uE3 into the reaction well.

c. Click **Print Assay** for a printout of the run. On a routine basis, your laboratory does not need a printed copy of the analytical run.

8. Click the **assay icon**. The screen goes to More Info about Assay window. The screen shows General Information about the run including test name, site

code, instrument ID, run ID, test date, kit lot, operator, and supervisor. There is also a comment area. Use this to enter comments regarding the assay. Click on right mouse for selection of fixed comments.

a. Click **QC Summary**.
   1) View the assay/tray median. The assay median is flagged if outside the aceptable limits. When flagged, NAPS MultiCalc status is yellow.
   2) View the summary of the SQC results. A "Fatal error from system controls" is shown when the NAPS MultiCalc status is red due to out of SQC results according to Westgard Rules. For each SQC, the window displays sample position, concentration (Resp), %CV, the assay average, the target value, and the +/- 2SD limits. Results are flagged * if outside 2 SD limits and ! if outside 3 SD limits. The same information is provided for the TQC (Control 4).

b. Score the run by clicking onto one of the three colors, red for prevent, yellow for held, or green for release. Refer to the attached Table I, PNS Quality Control Action Chart at NAPS Laboratory, Assay Status, for a summary of MultiCalc and supervisor scores at the run level. To determine whether the run is red, yellow, or green, use the following rules.

   NOTE: Any time you override a Multicalc score at the run/tray/result level that does not already have a system comment, the **Please comment** window appears and ask you to provide a reason for overriding the Multicalc score. When the window appears, entering a comment is mandatory. If you click **Cancel**, the Multicalc score does not change. This prevents a run without flags from being AutoRelease when your laboratory determines the run/tray/result is invalid.

   1) Use SQC results and the assay median to score the run.
   2) Score the run using Westgard Rules applied to the SQC results. Apply the Westgard Rules to the pair of results, e.g., the two low system controls or the two high system controls.

      - Score the run "red" if one of a pair is >3SD limits or two of a pair are >2SD limits.

      - Score the run "green" if one of a pair is >2SD but within 3SD or all results are within 2SD limits.

   3) Score the run "yellow" if the assay median is outside acceptable range and the run has at least one full tray. Otherwise, score the run "green". MultiCalc will score the run

"yellow". You may score the run "yellow" if there is a reason you want additional review by GDL's QA system.
NOTE: **Do not use tray median** for scoring PNS runs/trays. The analytes concentrations change with gestational age. Therefore, tray medians are not true indicator of an analytical shift.

4) Score the run "red" if during shutdown the AutoDELFIA fails the washer probe performance check. Most often it is because a washer probe becomes block and fails to wash wells correctly during the run.
5) Score the run "yellow" is for any reason you want additional review by GDL's QA system.

c. Click **Save Changes**. The screen returns to the tray icons in their relative shelf positions.

d. Click the free comment area to enter comment for the run. When a run is out of control, perform remedial actions before proceeding with the next run. Use this space to document and enter an explanatory comment of the actions carried out by your laboratory. If applicable, you may also select a fixed comment. Fixed comments available are now specific for the run level and assay.

e. Click **View Control Plot** or **View Trend Plot** to view the control or trend plots. See Section III.

9. Click onto Tray 1. You must view the STD curve, determine if the STD or SQC need reevaluation, view the QC and trend plots, and review individual results before scoring the tray.

The screen shows the plate with the 96 well positions in the upper left corner. View the 96 well positions by one of the four selections under Well Color Type. Each selection has its own pull down menu for more choices. Color codes are defined by **Color Description**.

In addition, if you click **Well Text Type**, along with the color for each well, you will also see text, e.g., 1 in the well if rpt = 1. Click onto the drop down menu. Select Checkmarks, Repeat count, or Probe number. (Positives is not used for TMS.)

a. Click onto a well position from the **Tray View** screen. It is highlighted in white. The information on the right of the screen, **Result Information**, such as barcode, concentration, sequence

number, repeat count, etc. and **Result Status**, red, yellow, or green, are filled in for that result.

1) You can score the individual result by clicking onto a color bar.
2) Right click for dropped down menu. Select Barcode History for the testing history of the selected sample. (Can also click onto the **Barcode History** icon.
3) Select More Info about Result for complete detail information of the Selected sample.

b. To view the standard curve information, click onto the Tray 1 icon on The bottom left screen.
   1) Click **Standard Curve Table**. The screen shows the count for STD A (O STD) and concentrations for ED 20, ED50, and ED80. Other information includes the scale for the x and y axis, method of curve fitting, slope, and intercept. Nominal concentration, calculated concentration for each replicate and the average, % difference between the nominal and the calculated concentration, response (count) for each replicate, %CV for the response, and %CV for the concentration are all displayed for each STD.

      A single point may be deleted from calculation of the STD curve. That point will be appropriately flagged as an outlier.

   2) Click **Standard Curve** to view the shape of the STD curve. The x axis is the concentration and the y axis is the response (bound). The shape of the curve is not actionable. If it differs from previous curves, score the tray "red" or "yellow" based on what and why the shape is different.
      a) Use F3, Dual Plot, to see STD curves from prior runs.
      b) Use the up arrow to select a date.
      c) <ENTER>, the red curve is the one for the selected date. Can view multiple curves.
      d) Click F6, Table, to see the same information as clicking Standard Curve Table.
      e) <ESC>.
      f) <ESC>.
      g) F2 to Quit.
   3) Click **Cancel**.

c. Click **Worksheet View** to see the data in worksheet format. There are 8 columns:
   - Seq(uence number)
   - (Bar)Code - for identification/accession number of specimen
   - Conc(entration)

- Inst(rument) Flags - error messages from the system
- M(ulti)C(alc) Flags - QC error message
- (area for) Comment
- T(o) B(e) R(eported) - results release to GDL
- More - for information specific to the result, such as RPT = 1, 1st repeat.

Use the up/down arrows to scan the worksheet for Instrument and MultiCalc errors.

On the right of the screen, select to view the current tray or assay, all or flagged data, and the sample type to be viewed on the screen. Sample Type includes Patients, Proficiencies, References, Standards, Controls, and Averages.

Duplicate concentrations and the average concentration of each STD are listed along with the with the 3 system control results, 2 tray control results, and all patient results. Individual results may be flagged yellow or red, and the reason is listed in either Instrument Error or MultiCalc Error.

Individual results may be gray instead of black. "Gray" means read only. The supervisor cannot make any changes to gray results. A result is gray when a specimen is retested and it is not on the repeat list.

The worksheet can be expanded on the screen when, for example, you wish to see the entire comment you entered instead of just the first line. Right click anywhere on the result line, select **Line Height**, select **1**, **3**,or **5**. 1 is as it is, 3 is 3 lines wide, and 5 is 5 lines wide.

1) Click **Code** to see the Sample History for a summary of each time the specimen was tested.

   The screen shows all testing completed for the specimen by site, instrument, test date, run ID, test name, NAPS status, GDL status, number of repeat testing for the specimen (rpt), counts, concentration, and whether the result was released to GDB. On your screen, GDL status and GDB are always "none" and "no".

   a) Click on a result line, highlighted in blue. From the Status info, you will see the NAPS MC (MultiCalc) and Supervisor status for the run, tray, and result. On your screen, GDL MC and GDL Status will always be "none". From the Date and Operator info, you will see

instrument ID, date and time of analysis, name of analyst, date and time of review and release, and name of supervisor. On your screen, GDL related information are always "NA".

b) Click Assay from the bottom of the screen. Go to the drop down menu to select **Notes** or **QC Summary**. With **Notes y**ou will see the fixed and free text comment at the assay level for the assay of the result line clicked from the above step. With **QC Summary**, you will access the same QC Summary from clicking the assay icon.

c) Click Tray from the bottom of the screen. Go to the drop down menu to select **Notes**, **Standard curve**, or **Flags**. With **Notes**, you will see the fixed comment and free text comment at the tray level. With **Standard curve**, you will see the standard curve table. With **Flags**, you will see instrument error messages for the tray, e.g., reagent dispense error. If there are no comments or flags, "NA" is displayed.

d) Click Result from the bottom of the screen. Go to the drop down menu to select **Notes** or **Flags**. With **Notes**, you will see the fixed comment or free text comment at the result level. With **Flags**, you will see AutoDELFIA (Instrument) and NAPS MultiCalc error messages. GDL MultCalc is always "NA" on your screen. If there are no error or comment for the result, "NA" is displayed.

e) Enter an accession number into the Barcode input bar on the upper right corner of the screen to do a search of the testing history for any specimen. Enter accession date, -, check digit, -, sequence #, N (for type), -, year, -, lab site. Click **Training** if you want the search to include training runs. Click **Search** to begin the search.

f) Click **Print**. Select **Database report** or **Screen dump**. With **Database report**, you will print the top ½ of the screen with the testing history only. With **Screen dump**, you will print the entire screen.

g) Click **Close**.

2) Click **Re-Evaluate** to reconstruct a STD curve or swap SQC. The only reasons to use the reevaluate key are 1) STDs were put in the wrong order or in reverse order 2) the SQCs were incorrectly barcoded and therefore put in the

wrong order. Reevaluate only after you have physically verified the error.

NOTE: Do not delete a STD to reconstruct a different STD curve. An outlier function already exist in the software.

a) View the information on the screen. All the STDs and controls are listed under Description with Tray #, Original Counts, and New counts. (Original and New are the same until a change is made.)
b) Click **Swap Standards** to swap two STDs. You can only swap one STD for another, but, you can do it more than once. For example, if STDs were put in reverse order, you need to swap F for A, complete the cycle, go back and swap E for B. Repeat D for C.
c) Click **Swap Controls** to swap two SQC. Instead of SQC low, medium, high, SQCs were barcoded incorrectly as high, medium, low. Swap the high for low.
d) Click **Swap**. Action selected will appear under Pending Actions. Now the Original and New counts are not the same.
e) Click **Evaluate**. Answer Yes to the prompt, "This operation will change the assay data permanently. Do you want to proceed?"
f) Proceed to review and release.

3) To change the status of an individual result, click anywhere on the result line except Code and Comment. This brings you to the More Info about Result window. This gives detailed information on the screen about the individual result including the barcode, sample class (type of sample such as patient, controls, etc.), subclass, sequence number, well #, repeat count, AutoDELFIA error, MultiCalc error, comments, rack and position #, counts, dilution factor, and concentration.

Click onto a color bar on the right of the Results Status window to color the Supervisor Status bar for the individual result and Click **Save Changes**. As previously stated, if the run/tray/result does not have a system comment, the **Please comment** window appears for you to provide a reason for overriding a Multicalc score.

Refer to the attached Table III, TMS Quality Control Action Chart at NAPS Laboratory, Sample Status, for a list of the most

common error messages, reason for the message, and correct action to take.

a) Score an individual result "red" if the result for AFP, hCG, uE3, PAPPA, or HG1 is **<STD**. Upon repeat, score the result "green" and enter the fixed comment "confirmed low".
   When the result is not confirmed as **<STD**, score the result "red" and repeat.
b) Score an individual result "red" if it has an instrument error message **clot detected**. Also, score the companion markers (including Inh) for 2nd trimester specimens and companion marker for 1st trimester specimens "red". To score the companion marker(s) red
   i. Click on the result line to go to the More Info about Result screen.
   ii. Enter the comment, clot detected, on the result line. Highlight the comment and hit <Ctrl> <C> to copy.
   iii. Click the **Edit Within Run**. The screen will show the two companion runs. If the run is not reviewed and released, the screen shows "Location:Ready for review", if the run is, the screen shows "Location: Released, waiting for data transfer".
   iv. Highlight a run, hit <Ctrl> <V> to paste the comment and score the result red.
   v. Click **Save Changes**. (This saves the comment and score for the highlighted run.) Answer **Yes** when ask "Please note that these change will be written to data base and cannot be cancelled afterward. Do you want to proceed?". Click Close. This returns you to the More Info about Result screen (step i).
   vi. Highlight the second run, hit <Ctrl> <V> to paste the comment. To score a result from a run that has been reviewed and released, you must click **Restore Selected**. Answer **Yes** when ask "Do you want to restore the selected assay? The changes made so far will be saved". The screen gets "refresh" and the highlighted run is always the first on

the screen. **Highlight the correct run** and score the result red.

NOTE: Once a run is restored, the assay bar returns to the screen. Click on the bar and click C**omplete Review**. All previous changes were saved.

vii. Click **Save Changes**. (This saves the comment and score for the second highlighted run.)
viii. Click **Close**

NOTE: If you only want to copy and paste a comment, you **do not** need to restore a run that has been reviewed and released. Copy and paste to both companion runs and click **Save Changes**.

c) Score an individual result "red" if the result for AFP, hCG, uE3, PAPPA, or HG1 is **>STD**. If uE3 is >STD, also score the AFP, HCG and Inhibin result "red".

d) Change the two red flags for a specimen that was repeated due to >STD. (The two samples are flagged red due to same barcode and not >STD. Both situations are occurring and the error with the higher priority is listed.)

For repeats when AFP, hCG, or HG1 is >STD, scored the diluted sample "green" if it is confirmed high. Enter the fixed comment ">STD diluted 1:5". For the undiluted sample, score "yellow" for GDL's QA to disable. Enter the fixed comment, "confirmed high".

For repeats when PAPPA is >STD, there is only one flag for >STD. Score the undiluted sample "green" if it is a confirmed high.

For repeats when uE3 is >STD, scored the diluted and undiluted results "yellow". Use the fixed comment "confirmed high" for the undiluted sample and ">STD diluted 1:5" for the diluted sample.

Scoring as described will keep the specimen off the Local Repeats list.
NOTE: If the counts for any analyte is still beyond the highest standard after a 1:5 dilution, send the maternal serum specimen to GDLB under cold storage.

e) Do not change the 2 red flags for the two specimens with the "same barcode" due to a barcoding error. The two specimens will receive new barcodes before repeat testing. Staff with Security Level 3 or Wallac staff will delete the original accession number from the Local Repeat list. On repeat testing you MUST enter into the free comment area for both specimens that the accession number is the new accession number for the two specimens which had the same barcode. Enter the following free comment: new accession number for (the original accession number) for both specimens. Use the free text comment box for the tray to enter the cause of the error.

f.) Score an individual result "red" if it has the following Instrument error messages. MultiCalc will score these results "red".

- Too little liquid
- Liquid loss detected
- Liquid surface unstable
- Liquid moving failed
- No liquid found
- Invalid barcode, i.e., any barcode without the capital P such as barcodes generated by the newborn screening barcode printer or key enter manually without the capital P.

g) Score an individual result "red" if the instrument error message is **Possible foam detected**. Check the dilution strips. If the diluent level is visually higher than in adjacent strips, a used strip may have been used.

h) Score an individual result "green" if the error message is **Barcode rack**. Check the worklist to verify that the results are in the correct sequence. MultiCalc will score these results "yellow". This occurs when the barcode on the sample rack cannot be read. For the 12 samples

in the rack (or less if partial), in the main, you will see only 3 results with the error message. As the 4 probes sample simultaneously, only the result sampled by the 1st probe will contain the message. When rack #1 is affected, you will see 4 error messages. As pos #1 of the rack is sampled, other probes are still sampling SQCs or STDs. Score "yellow" if you want GDL to review.

4) Click **Comment** to enter comment regarding the sample or right click to select a fixed comment. Fixed comments available are now specific to the sample level and assay.
5) Click **Print Tray** to print the results of the tray when needed.

d. View control and trend plots from this screen. See Section VII.B.
e. Click on the Tray 1 icon on the bottom left to enter status of the tray. The more Info about Tray window is displayed on the screen.

Click onto a color bar on the right to color the NAPS Supervisor Status bar. Score the tray by applying the Westgard Rules to the pair of TQC results.

Refer to the attached Table II, TMS Quality Control Action Chart at NAPS Laboratory, Tray Status, for a summary of MultiCalc and supervisor's scores at the tray level.

1) Score the tray **red** if one of the bracketing TQC result is >3SD limits or two are >2SD but within 3SD limits.
2) Score the tray **green** if the two bracketing TQC results are within 2SD limits or one is >2SD limits but within 3SD.
3) Score the tray yellow if you want additional review by GDL's QA system.

The software scores the run status using SQC results with tray status using TQC results. Repeat the test for every specimen on the prevented run/tray for the single analyte.

f. Click **Comment** to enter comment regarding the tray or right click to select a fixed comment. Fixed comments available are now specific to the tray level and assay.
g. Click **Save Changes**.
h. Click **Accept Tray**.

10. Click on Tray 2 icon.
    a. To view the STD curve data, click onto the Tray 2 icon on the bottom

left screen. Click **Standard Curve Table**. Tray 2 contains 2 replicates of STD B and E for AFP and uE3 and 2 replicates of STD C and E for hCG and PAPPA. A "corrected" STD curve is constructed to quantitate specimens on Tray 2. "Corrected Ref curve used in Standardisation" is on the screen. The "corrected" STD curve is constructed using the new responses for STD B (or C) and E along with the original responses from Tray 1 for the remaining STDs. The same information is shown for the "corrected" curve as was for the original curve. Review the "corrected" STD curve data. The screen shows the two replicates and the average for STD B (or C) and E. The calculated response for the remaining STDs off of the "corrected" curve is listed under Response. This can be compared to the measured response used to construct the original curve. The % listed under %dRef is the movement of the STD from the original curve to the "corrected" curve. Response for STD A for AFP, hCG, and PAPPA will not change from tray to tray but will change for uE3.
NOTE: The HG1 assay has 6 standards on Tray 1 only. Subsequent trays contain 2 standards.

b. Click **Standard Curve** to see the shape of the STD curve. Use Esc and F2 when done.
c. Click **Cancel**.
d. Click **Worksheet View**. Scroll down the tray to review the results for flags and score as for Tray 1.
e. Score Tray 2, click **SaveChanges/Accept Tray**.

11. Continue until all trays for the assay have been reviewed. For the last tray which contains the probe controls, score the tray using the two TQCs bracketing the patient specimens. Override the MultiCalc score since it scores the tray using every TQC result. If one or more probe control is >3SD, score the run **red** and repeat. If one or more probe control is >2SD, change the **green** score on each tray to **yellow** for GDL review. For example, if the two bracketing TQC are >2SD, score the tray red and change all other green trays to yellow or if the first probe control is >3SD, score the run red.

    Determine the probable cause why the probe control results are outside the 3 and 2 SD limits. The investigation must include but is not limited to examining the TQC tubes, reagent tips, dilution strips, and viewing the pipet map and the history browser. Use the free comment section for the tray to enter the probable cause.

    After a tray has been reviewed, the box changes from the background gray color to a lighter gray color box.

12. Click **Complete Review** when all trays have been reviewed and scored.

Access to it is only after all trays are scored. The screen goes back to All AutoDELFIA Assay window. Released runs are placed in an electronic mail box for automatic transmission to GDLB's QA system where results are released to SIS.

13. Click **Cancel Review** to leave without making any changes to the data.

14. Select the next run to review and release. When all runs are reviewed and released, click Close.

B. REVIEW CONTROL AND TREND PLOTS

1. Click **View Control Plots**. This allows you to review the quality control charts. You are now in MultiCalc and the mouse does not work within MultiCalc. Use Fl for a plot of the low SQC. On the bottom of the screen are laboratory site, AutoDELFIA ID, run #, and test date. Use the right and left arrow to move the pointer from point to point. The numeric value of the point is listed as "Value = ". The incremental step of the pointer is defined by "Step = ", i.e., if Step = 1, then the pointer moves from one point to the next. Use the up and down arrow to change the step the cursor steps over the plotted points. Other parameters include the target, mean (mean calculated from all plotted data points) with SD, CV, and slope.
    a. Use the left/right arrow to move to the date when a change was made, i.e., reagent lot change or QC limit change, to set the beginning of the window, then use the F1 key. Use F3 to recalculated the mean using data starting with the date of change. Likewise, move to the date of choice, F1, move to a second date, F2 to set a window. Use F3 to calculate the mean for the window.
    b. Click F7 to print the QC plot.
    c. Click F5 to delete QC data points from an out of control run. Click F5 again to add the data point back.
    d. Click ESC.
    e. Use F2 for a plot of the medium SQC and proceed as above.
    f. Use F3 for a plot of the high SQC and proceed as above.
    g. Use F4 for a plot of the TQC and proceed as above.
    h. Click ESC twice to exit Multi Calc.

2. Click **View Trend Plots** to see a plot of the other assay parameters. Parameters monitor by GDL QA includes ED50(F2), slope(F5), intercept(F6). Click F8 twice to access two other parameters of interest, Trend 1 (F1) and Trend 5 (F5). This is for your information only.

   These parameters are not actionable. Hit ESC twice to exit Multi Calc.

C. REPORTS

1. Click **Routine Program/Result Viewer/Reports**.

2. Click **Repeats requested locally** or **Repeats requested by GDL**.

3. Click **Print**. View the repeat list. Click the **printer icon** to print the repeat list. Click [X], [X] to close the window.

4. Click **Close**.

D. RESTORE A RUN

Use this selection to make corrections to runs which have been reviewed and released but not yet sent to GDLB's QA system.

1. Follow daily routine procedures and click onto the laboratory icon.

2. Click **Restore Non-Posted** on the bottom of the right screen. All assays, previously reviewed and released and not yet picked up by the GDL's QA system, are shown on the screen. When restored, all prior changes and status made by the supervisor are retained. However, when a run is in "restored", the prior Local Repeats list is not retained. A new Repeats Requested Locally list is generated when Complete Review is clicked.
    a. Select an assay by clicking onto the assay bar. A warning shows up in the comment area, "Warning! You are about to restore one (or the # of bars clicked) assay".
    b. Click Restore Selected.
    c. Click Close. Proceed to review and release.

E. VIEW ARCHIVE DATA

1. Follow daily routine procedures to logon. Click **Routine Program/Result Viewer**. Select **Selected date (read-only)**. This is read only and no changes can be made to the data files.
2. Click on the drop down menu for a calendar and select a date. Calendar goes to today's date. Use the forward or backward arrow to change the month. Or, click **Advanced** for the year's calendar and select a date.
3. Click onto laboratory icon, view the run, then click **Close**.

F. TO VIEW THE PIPETTING MAP

1. Use the AutoDELFIA PC and click Options/Pipetting map. You will see the pipetting map information for the current or last run from the AutoDELFIA.

2. View the screen. On the bottom left is the plate # and analyte. All 96 wells from the plate are displayed. Each well shows the sample probe tip #, sample rack # (or dilution rack # for hCG), position #, and counts. If there were a pipetting error for a particular sample, that particular well will show "E". Click the well and information regarding the sample will appear on the screen. Click message for the specific error message.

3. Click the left arrow (top left of screen) to see a preceding plate and the right arrow to see the next plate.

4. Click the File Folder icon (top left of screen) to view a previous run. The screen displays the "Previous runs" window. Select the run by date and click OK.

5. Click Exit to leave.

G. BACKUP PROCEDURES

PNS (AFP, hCG, uE3, Inh, PAPPA, and HG1) and NBS run data are backed up every day to the internal DLT drive installed on the server. Backup procedures store all run data on DLT tape for emergency retrieval. Four tapes, labeled as Tape #1, #2, #3, and #4, are used. One tape is used for one week. Rotate the use of the tapes so that the order of use is #1, #2, #3, #4, #1, #2, #3, #4, etc. Even though backup is done every day, user intervention is required only once a week, e.g., every Friday.

1. Full backup
   Do this at the server weekly, e.g., every Friday. Click **Start/Full Backup**.
2. Incremental backup
   Incremental backup is performed daily without intervention/action from the user. It is run automatically every night and it backs up all changed data from the file server to the tape. Therefore, keep the tape loaded in the DLT tape drive. No printed report will be generated from this procedure.

H. MANUALLY DOWNLOAD A RUN TO THE SUPERVISOR'S PC

1. Press the <ALT>, <TAB> keys simultaneously to MultiCalc AutoDELFIA. The MultiCalc California TMS screen is displayed. Counter is on the screen.

2. Move arrow to 1235 Fluoro and <Enter>.

3. Press F6 (recover).

4. Use the up and down arrows to select a run.

5. Press <ENTER>, <ENTER>.

6. Wait for the screen to show "counter". The screen shows "counter" after the run was downloaded.

7. Move arrow to 1235 Fluoro and repeat for another run.

8. <ALT>, <TAB> to AutoDELFIA workstation.

I. IMMEDIATE TRANSFER OF DATA TO/FROM GDLB

1. Logon, click **Transfer to GDLB** to transfer specific reviewed and released runs to GDLB's QA system. Also click **Transfer to GDLB** at GDL's request to receive immediately the Repeats Requested by GDL list or changes made to the assay protocol that your laboratory need prior to the next electronic mail exchange.

   Routinely, reviewed and released runs are downloaded to GDLB's QA system each night at a specific time. Also as a routine, if the day's run is completed by 3:00 pm, use **Transfer to GDLB** to download the run that afternoon.

   Clicking **Transfer to GDLB** downloads a run immediately to GDLB. Other than routine, use **Transfer to GDLB** only at GDL's request.

2. Click **Transfer to GDLB** to download/upload data immediately to/from GDLB. Select what you need to download/upload by clicking **Transfer All Data**, **Transfer TMS Data**, or **Transfer NBS/MSMS Data**.

J. LEVEL 3 ACTIVITIES

1. Delete Specimens from Repeat List
   a. Ctrl, Alt, Delete to logon.
   b. Enter user name and password. To perform this step, you must logon at Level 3.
   c. Click **Management/Repeat Manager**.
   d. View the screen. On the right side of the screen are the selections. Select **Prenatal Assays**, **Screening Assays** and/or **Training Assays**, **All** for instruments, and **Clear Mode**.

      Any selected run with repeats on the Local Repeat list will show up in the Repeat Manager window by analyte, date, instrument ID, and run #.
   e. Click onto the run which contains the repeated specimen(s) you want deleted. All specimens will be listed in the lower window by Assay, Tray

#, Barcode, Repeat Type, and Notes.

f. Mark the specimen(s) you want deleted. Click Cleared Selected. A prompt appears on the screen, "You are about to clear " X" repeats. Please confirm". Click OK. "X" represents the actual number of specimens marked. Click Restore Selected if the Mode to Use is Restore.
g. Click **Exit**.
h. Click **Exit/Log Off**.

2. Print QC/Trend Charts Easily
   a. Logon as Level 3.
   b. Click **Management/Multicalc QC Report**.
   c. Select the **AutoDELFIA Panel** tab. You will see every NAPS laboratory and GDL listed. You will also see every instrument listed. Your laboratory has access to only your own laboratory data and only your own instrument data.
   d. Select the instrument by checking the box next to your laboratory/instrument. (You have to check the box. Otherwise, it does not work.) If you check your laboratory, you will see the system and tray control data for all systems in your laboratory by panel and test. If you check one of your instruments, you will see the system and tray control data for just that one system by panel and test.
   e. Select Report Format, either **Graphical** or **Table**.
   f. Select QC Printout, either **Control**, **Trends**, or **Both**.
   g. Select an assay from **Available Tests**. Click **Add** to add to **Selected Tests**.
   h. Select Date Range **From** mm/dd/yy and **To** mm/dd/yy.
   i. Click **Print**. If **Graphic** was selected, you will have a printout of the plotted data (typical QC chart) for each of the three system controls and the tray control. If **Table** was selected, you will have a printout of data listed by LAB, (Instrument)ID, DATE, VALUE, TARGET RANGE, AND % DIFF(erence from target). If you made a choice that is inconsistent, you will get an empty page with a heading only.
   j. Click **Close**.

VIII. CALCULATIONS

Refer to the protocol named in Section III.

IX. REPORTING RESULTS

Refer to the protocol named in Section III.

X. PROCEDURE NOTES

NA

XI. LIMITATIONS OF PROCEDURE

NA

XII. REFERENCES

Refer to the protocol named in Section III.

Appendix 5L

TABLE 1
PNS Quality Control Action Chart at NAPS Laboratory

A. Assay Status

PC MultiCalc Status	QC Results	NAPS Laboratory's Actions
Prevented	1SC>3 SD 2SC (same level)>2 SD	Score the run red. Determine the source of error, take corrective action and repeat the run. If the error is due to switching two standards or system controls, reevaluate the run. Follow the Westgard rule to score the reevaluated run.
Hold	1SC > 2SD Assay median is outside acceptable limit Standard curve was edited	Score the run green if 1 SC>2SD and all other QC parameters are within limits. Score the run yellow if 2 or more SC>2SD and other QC parameters indicate the run may have a bias. Score the run red if the assay median is outside acceptable limits. Score the run red if 2 or more STDs with both replicates were outliers
Release	All QC parameters are within limits	Score the run green. Score the run red if the system failed the washer test.

B. Tray Status

NAPS MultiCalc Status	QC Results	NAPS Laboratory's Actions
Prevented	1 of the bracketing TQC is > 3 SD Both bracketing TQC are > 2SD Bracketing TQC violate R4S Rule Less than two TQC on the tray	Score the tray red. Determine the cause, take corrective action and repeat the tray.
Prevented	1 or more probe control is > 3SD 1 or more probe controls are > 2SD or 1 TQC and 1 Probe control >2SD	Score the run red. Determine if a sample probe is plugged. Clean the probes and repeat the run. Score the tray yellow and change the status of each **green** tray to yellow .
Yellow	1 TQC is > 2 SD. 1 probe control >2SD	Score the tray green. Score the tray yellow and change the status of each **green** tray to yellow
Green	All TQC and probe controls are within the limits	Score the tray green.

C. Sample Status

NAPS MultiCalc™ Status	AD/MC Error Message	Cause	NAPS Laboratory's Action
Prevented	Too little liquid	Sample probes detected liquid surface close to the Z max for liquid level. This is a warning that the full volume of sample may not have been aspirated.	Score the result "red". Check sample tube. If liquid level is >350uL for 1st or 2nd trimester specimen, repeat. If <350uL, call specimen inadequate. Security Level 3 person will delete specimen from the Local Repeat list.
Prevented	Possible foam detected (hCG, PAPPA, HG1)	Sample probes detected liquid surface higher than expected level.	Score the result "red" and repeat. Check dilution strips. If liquid level is visually higher than adjacent positions/strips, analyst failed to dump used strips during shutdown. Enter comment during R&R. Take steps to prevent such errors.
Prevented	Liquid loss detected (hCG, PAPPA, HG1)	AutoDELFIA detects less liquid than it expects in the dilution strips	Score result "red" and repeat. Check dilution strips. If liquid level is visually lower than adjacent positions, check for leaks, cracks on bottom strips, liquid in rack. Clean rack. Enter comment during R&R. If no signs of leaks and it persists, call Proxy.
Held	Liquid surface unstable	The difference between the established liquid level and the liquid level detected during aspiration is more than the set limit.	Score result "red" and repeat. Check specimens for air bubbles. Enter comment during R&R. If it persists, call Proxy.
Prevented	Liquid moving failed	Sample probe was unable transfer liquid from the source to the target	Score the result "red" and repeat. Check liquid level in Stds, samples, dilution strips. Check history browser for specific reason. Enter comment during R&R. Call Proxy if it persists.
Prevented	No liquid found	Sample probes were unable to detect liquid at Zmax for search level.	Score the result "red". Check sample tube. If liquid level is >350uL for 1st or 2nd trimester specimen, repeat. Call Proxy. If <350uL, call specimen inadequate. Security Level 3 person will delete specimen from the Local Repeat list.
Prevented	Clot detected	After sampling, the probes move to a position above the originally detected liquid surface. If probe detects it is still in contact with the sample, it assumes the sample has a clot.	Score the result "red" for quad marker for 1st or the two analytes for 2nd trimester specimen. Check tube for clots and remove. Mix, centrifuge and retest for all three analytes. If clot detected message is found on control samples and it persists, call Proxy.
Prevented	Duplicate barcode	The system has detected that a tube with the same barcode ID has already been scanned and loaded in the sample loading process.	1) Error message due to barcoding error. Score results "red" and repeat per Protocol. 2) Error message when repeat testing for result >STD Score both results "yellow" for uE3, if confirmed >STD, score "green" for AFP, hCG, PAPPA, or HG1. Enter fixed comment, confirmed high for the undiluted sample and >STD, diluted 1:5 for the diluted sample. If

			unconfirmed, score both results “red” and repeat.
Held	Barcode rack	During sampling, the barcode on a sample rack cannot be read.	Score the results “green”. Check barcode and replace or clean as needed.
Prevented	<STD	A result was less than the set lower concentration limit.	Score the result “red” and repeat. If result is a repeat and is a confirmed low, enter fixed comment and score result “green”. If unconfirmed low, score result “red” and repeat.
Prevented	>STD	A result is greater than the concentration of the highest STD.	Score the >STD AFP, hCH, PAPPA, or HG1 result “red” . Score the results for all three analyte “red” when uE3 is >STD. Repeat per protocol.